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Cutaneous Uptake of ^{14}C-HD Vapor by the Hairless Guinea Pig

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13. ABSTRACT (*Maximum 200 words*)

The hairless guinea pig (HGP) is used by our laboratory to model the human cutaneous response to sulfur mustard (HD) exposure. We have determined the HD content in the skin of HGP after 7-minute exposures to vapors saturated with a mixture of HD and ^{14}C-HD. Concentration/time (C_t) values in the range of 2 mg/cm^2/min were determined by counting skin ^{14}C disintegrations per minute (dpm) in animals euthanized immediately after exposure. These values are similar to human penetration rates obtained by other investigators. A direct relationship between C_t and relative humidity was demonstrated in 5 of 6 studies. A rate curve monitoring the reduction in skin ^{14}C dpm was developed for animals euthanized between 0 and 24 hours post exposure. This curve showed the greatest change after 1 hour. Epidermal and dermal distribution of ^{14}C at 24 hours was measured for two animals. Site preference for HD penetration, multiple use of a vapor cap containing HD, and ^{14}C content of adhesive tape were also investigated with radiolabeled HD to evaluate other aspects of the experimental model. These results contribute to a better understanding of the cutaneous response to HD in the HGP model.

14. SUBJECT TERMS
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INTRODUCTION

The use of sulfur mustard (2,2'-dichlorodiethyl sulfide, HD) in recent military conflicts such as the Iran-Iraq war and the continuing threat of its use in future conflicts have intensified research efforts to develop effective therapy for the prevention and treatments of HD-induced vesicant injury. Decision Tree Networks (DTNs) outlining research management strategies for evaluating the efficacy of compounds against chemical warfare agents have been developed for potential antivesicant and topical treatment compounds. These DTNs include specific *in vitro* and *in vivo* models for the purpose of screening classes of compounds.[1]

During the last several years considerable effort has gone into establishing the hairless guinea pig (HGP) as an *in vivo* model of vesicant injury. Systematic studies have been conducted to show a correlation between HD vapor exposures and microblister formation in the HGP. Cutaneous exposure of the HGP to HD vapor produced lesions that varied in severity depending on the total time of vapor exposure.[2-4]

Although these studies demonstrated the suitability and versatility of the HGP as a vesicant model, they were carried out without the knowledge of the actual concentration of vesicant in the skin. This information on concentration has been obtained for other animal models.[5,6,7] Renshaw[5] summarized the results of a number of human skin studies with values ranging from 1-4 $\mu g/cm^2$/min for the rate of penetration of liquid or saturated mustard vapor at 70°F. Henriques et al.[6] measured penetration rates of liquid sulfur mustard for men, pigs, and rabbits using ^{35}S labeled HD to determine the amount fixed in the abdominal skin after a one-hour exposure at a variety of environmental temperatures. He obtained values at 60°F of 2.2, 0.67, and 6.0 $\mu g/cm^2$/min respectively for these animal models. Bergmann et al.[7] measured the penetration rate of sulfur mustard vapor applied to human forearms by titrating the HCl liberated upon complete hydrolysis of the vesicant. He found that mustard vapor penetrates into forearm skin at a rate of 1.4 $\mu g/cm^2$/min at temperatures of 70-73°F and 44-46% relative humidity and that the rate held constant for exposure times of 3 to 30 minutes.

This study was designed to measure the cutaneous uptake of saturated sulfur mustard vapor in the hairless guinea pig using ^{14}C-labeled HD. We followed the procedures described by Mershon et al.[3] Our objectives were 1) to determine the initial uptake of mustard in the skin following a 7-minute exposure and from this data determine penetration rates; 2) to determine uptake of HD by measuring skin concentrations at 1, 3, 6, 12, and 24 hours after exposure; and 3) to examine the influence of relative humidity and temperature on penetration rates.

MATERIALS AND METHODS

1. Animal Preparation. Male [CrL:IAF/HA(hr/hr/BR)] euthymic hairless guinea pigs weighing 250-400 g were used. The general procedures used in this protocol were described by Mershon et al.[3] The back of each animal was carefully wiped with 70% isopropyl alcohol solution

the day before exposure to remove soil and debris. On the day of the experiment the animals were transferred from the quarantine room to the laboratory in polycarbonate cages. The exposure areas were outlined with a 8-cm x 12-cm template that was centered over the animal's back. The animals were weighed and anesthetized with a combination of ketamine hydrochloride (30 mg/kg) and xylazine (6 mg/kg) i.m.[8] Each animal was draped with polyethylene-backed absorbent sheeting (Kaydry, Kimberly Clark, Roswell, GA). Tape assemblies were prepared by affixing double-sided vinyl tape (Devoseal, Devon Tape Corp., Carlstadt, NJ) placed edge to edge on vinyl-coated colored tape (TimeMed Labeling Systems, Inc., Burr Ridge, IL). A cork borer centered over the removable vinyl tape covering was used to punch through both tapes. The colored tape was trimmed and a free edge was adhered to a pull tab. The punched adhesive assemblies were applied edge to edge (colored side up) and parallel to the spine on each side of an animal's back. This procedure produces two rows of exposure sites. Each exposure area was marked (permanent marker) with dots at the outermost edges of the hole in the tape assembly. After assemblies were adhered to the skin, protective coverings were removed with forceps to expose fresh adhesive surfaces.

2. Isotope preparation. The HD employed in this study was a preparation of 95%(v/v) lot #HD-U-4244-CTF-N-1 (US Army Edgewood Research, Development and Engineering Center, Aberdeen Proving Ground, MD) and 5%(v/v) lot #39-132-2B (US Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD. Lot #HD-U-4244-CTF-N-1 was 97.6 mole% HD and lot #39-132-2B was 75 mg/ml HD in benzene with a ^{14}C HD activity of 2 x 10^7 dpm/μl. The preparation contained 93.2% HD(v/v) with a ^{14}C activity of 1 x 10^6 disintegrations per minute(dpm)/μl (2.22 x 10^6 dpm = 1 μCi).

3. HD Exposure. Exposure to HD vapor was accomplished using the methods descibed in SOP 91-067-DB-01, "Surety Procedures for Cutaneous Applications of Sulfur Mustard (HD) on the Skin of Laboratory Animals." Briefly, ten microliters of the preparation were pipeted onto filter paper discs (14 mm dia., Whatman # 2, Whatman Inc., Haverhill MA), attached to the inside top surface of polyethylene caps 14 mm inside diameter and 5 mm deep (No. P799C, Columbia Diagnostics, Inc., Springfield, VA). The quantity of HD was sufficient to completely saturate the filter disc without run-off. Following a 5 minute equilibration period, the caps were adhered sequentially to the adhesive assembly over the eight exposure sites (12-mm diameter holes in tape assembly). Forceps were used to apply or remove caps (ending exposures) and to remove tape assemblies from skin. After vapor exposures, the guinea pigs were maintained in the respective polycarbonate cages for specific time intervals until euthanization. Anesthatized animals were euthanatized with 2 ml cardiac injections of sodium pentobarbital (64.8 mg/ml) at chosen intervals up to 24 hours following exposure. Skin punches were taken following euthanasia. Animal carcasses were disposed of in accordance with SGRD-UV-VM SOP No. 14 "Animal Euthanasia" dated 26 February 1991. The skin over the dorsal, thoracic-lumbar area was removed. Dermal punch (14 mm) specimens were immediately taken from the center of all animal exposure sites. Exposure sites were identified and collected as shown in Figure 1.

4. Processing of samples. Skin punches were placed inside 20 ml glass scintillation vials[9] containing 1 ml of tissue solubilizer, Beckman BTS 450, Beckman Instruments Inc., Fullerton, CA, and processed as follows.

a. Added another 1 ml of tissue solubilizer and heated at 55^{0}C for 4 hours.
b. Cooled to room temperature, added 100 ul of 30% hydrogen peroxide and heated at 55°C for 30 minutes.
c. Cooled, added 10 ml of Beckman Ready Safe scintillation cocktail, vortexed, added 100 μl glacial acetic acid, and vortexed.
d. Centrifuged vial at 2500 rpm for 15 minutes.
e. Determined ^{14}C content with a Beckman Liquid Scintillation System, 5801 Series.

The double-sided adhesive tape that held the vapor cup to the HGP was processed and measured for ^{14}C content in the same way as the skin punches. Following the 7-minute exposure, each vapor cup was placed in 10 ml methanol, and analyzed for HD content with gas chromatographic flame ionization detection and for ^{14}C content with a liquid scintillation counter.

5. Isotopic assay standardization. A series of 8 standard curves in Figures 3&4 was prepared to investigate addition and recovery during sample processing. Each curve represented a step in sample processing and contained 8 concentrations which were prepared from a stock solution of 10 μl ^{14}C HD in 10 ml of methanol, Fisher ACS Grade, Fisher Scientific, Pittsburgh, PA. The following describes the contents and procedures prior to dpm determination.

a. Methanol solutions
b. Methanol solutions + Tissue solubilizer
c. Methanol solutions + Tissue solubilizer + H_2O_2
d. Methanol solutions + Tissue solubilizer(Digest)+ H_2O_2
e. Tissue + Tissue solubilizer(Digest) + H_2O_2 + Methanol solutions
f. Tissue + Tissue solubilizer + Methanol solutions(Digest) + H_2O_2
g. Tape + Tissue Solubilizer(Digest) + H_2O_2 + Methanol solutions
h. Tape + Tissue solubilizer + Methanol solutions(Digest) + H_2O_2

6. Chromatographic methodologies. Gas chromatographic flame ionization detection (GCFID) measurements (Hewlett Packard, Model 5890, Rockville, MD) were made on ^{14}C HD methanol solutions. A 30 m, 1.5 μm DB-1 column with a 0.53 mm diameter was used for separations. The GC measurements corroborated the presence of HD and consistency of preparation. Thin layer chromatographic(TLC) measurements were made on the ^{14}C HD preparation using a 5%(v/v) methanol in chloroform solvent system with a 250 micron silica sheet. TLC analysis was made to confirm HD purity. A Berthold Automatic TLC-Analyzer LB 283 (Berthold Instruments Inc., Pittsburgh, PA), was used to detect the ^{14}C HD.

7. Correlation Study. For this study, 52 animals were used . The animals were exposed to vapor HD (see section IV,A.3) for **7 minutes**. Each group of animals was prepared for skin

punches at the following time points: 0 (n=17), 1 (n=6), 3 (n=6), 6 (n=6), 12 (n=6), and 24 (n=11) hours. This study investigated relationships between HD content of cutaneous HGP samples and 1) time after exposure (Figure 2), 2) humidity (Table I), and 3) temperature (Table I).

8. Data Analysis Plan. Dpm data from skin punches (Table II), tape (Table III), and solution (Table IV) were statistically evaluated for mean, standard deviation, and standard error of the mean by Lotus 1-2-3, release 4.01, (Lotus Development Corp., Cambridge, MA). Figures II, III, and IV were created with Sigma Plot version 2.01 (Jandel Corp., San Rafael, CA). Sigma Stat, version 1 (Jandel Corp., San Rafael, CA), was used (1) to determine differences in addition and recovery curves, and (2) to evaluate data from multiple applications of an individual vapor cap.

LABORATORY ANIMAL PROCEDURES

1. Animals required. A total of 52 male [CrL:IAF/HA(hr/hr/BR)] euthymic hairless guinea pigs (Cavia porcellus) were used. Upon arrival to USAMRICD, they were quarantined and screened for evidence of disease before use. They were maintained under an AAALAC accredited animal care and use program in plastic cages (Lab Products, Inc., Maywood, NJ). The guinea pigs were housed in groups of two, on contact bedding (Cellu-dri, Sheperd Specialty Papers, Kalamazoo, MI) changed three times per week. They were provided commercial certified guinea pig ration (Zeigler Bros., Inc. Gardners, PA) and tap water ad libitum. Animal holding rooms were maintained at 21° ± 2°C with 50% ± 10% relative humidity using at least 10 complete air changes per hour of 100% conditioned fresh air. All cages were covered to minimize heat loss, and animals were maintained on a 12-hour light/dark full spectrum lighting cycle with no twilight.

2. Animal procedures. Guinea pigs were anesthetized with intramuscular doses of a combination of 30 mg/ml ketamine HCl (Vetelar, 100 mg/ml; Parke-Davis, Division of Warner-Lambert Co., Morris Plains, NJ) and 6 mg/kg xylazine (Rompun, 20 mg/ml; Mobay Corp., Animal Health Division, Shawnee, KS) during exposure. Injections were administered into the lateral thigh using a tuberculin syringe with a 25- to 27-gauge needle. Guinea pigs were manually restrained by trained personnel while they were anesthetized and examined. Anesthetized animals were secured to a restraining board in sternal recumbency during exposure and treatment. After exposure the animals were housed in polycarbonate shoe box cages in a fume hood until they were euthanatized. All cages were covered with plastic-backed absorbent pads to minimize heat loss. On the day of exposure, the guinea pigs were maintained in individual polycarbonate shoe box cages stationed in the exposure hoods. Food and water were offered to the animals while they were maintained in the hood.

RESULTS and DISCUSSION

Absorption at zero time. The disposition of radioactivity following vapor cap application of sulfur mustard is shown in Table I. The concentration/time value, C_T, for the 7-minute exposure

was calculated from the zero time experiments in each of the studies. Zero time experiments were those in which the skin samples were harvested immediately following exposure. C_T was calculated from the equation:

$$C_T = \frac{9.32\ \mu l \times 1.27 \times 10^3 ug/ul \times (\text{Tissue DPM}/\text{Theoretical DPM})}{3.14 \times (0.7)^2\ cm^2 \times 7\ min}$$

where 10.0 μl of a preparation containing 93.2% HD(v/v), density 1.27 g/ml, were added to the vapor cap, tissue DPM** are taken from Table II under ZERO time, theoretical DPM** are maximum available DPM, Table IV, in vapor cap prior to exposure, the radius of the skin punch is 0.7 cm, and the time of exposure is 7 minutes. Tissue DPM were corrected for losses in sample processing as described in "Addition and Recovery." Tissue and Theoretical DPM were background corrected; background was 38.2 dpm.

Connection between this study and historical data. Bergmann et al.[7] determined the penetration rate of saturated vapors of sulfur mustard to be 1.4 $\mu g/cm^2/min$ at 70-73°F and 44-46% relative humidity for exposure times of 3-30 minutes on human forearms. Henriques et al.[6] obtained an average penetration value of 3.7 $\mu g/cm^2/min$ following a 1-hour exposure of liquid H to human abdominal skin at 72°F. Henriques et al.[6] also obtained average penetration rates for man, pig, and rabbit of 2.2, 0.67, and 6.0 $\mu g/cm^2/min$, respectively, following 1-hour exposures of liquid H to abdominal skin at 60°F. The C_T values in the present study are consistent in magnitude with these previous human values and demonstrate the suitability of the hairless guinea pig model in mustard studies.

Absorption change with time. Figure 2 is a rate curve that shows the 24-hour time course for the disappearance of ^{14}C-HD from skin tissue with points measured at 0,1,3,6,12 and 24 hours post exposure. The rate curve shows the largest change occurring in the first hour with smaller decreases thereafter. This curve represents the data collected from six studies. Table II contains the raw dpm data used to construct the curve. Two animals from the second study were euthanatized 24 hours after exposure, and we saw a significant decrease in the activity of their skin samples. The remaining studies were used to investigate this decrease in ^{14}C activity over 24 hours. Offgassing studies[10] have shown that following HD vapor cup exposures to weanling pigs, HD can be detected on the exposed skin of the animal up to 6 hours following exposure. Offgassing from the hairless guinea pig could contribute to the decrease in activity seen in the rate curve. The process could also be delivery, penetration, fixing or covalent binding to protein, and uptake of the unbound HD by the animal.

Epidermal and dermal data verifies binding has occurred. Eight (14mm) skin punches were taken 24 hours after exposure from animals #115 and #116. The epidermal and dermal layers were separated, and the ^{14}C content of each was determined. The epidermal and dermal layers contained 214(±26)* DPM and 130(±6) DPM respectively. A high salt buffer was employed in the epidermal-dermal separation.[11] Eight high salt buffer solutions used in the

separations contained little ^{14}C 59(±2) DPM pointing to significant binding of HD to the epidermal and dermal layer at this point.

Standard error of the Mean, SEM, is in parenthesis.

Analysis of temperature and humidity. Studies I through V (Table I) indicated that C_T increases with increasing relative humidity. Renshaw[12] and McAdams[13] reported an increase in skin damage with increasing moisture on the skin. An increased uptake as indicated by a larger C_T agrees with their skin damage observations. However, the results in study VI do not support the trend seen in the five previous studies. That is, the C_T of 1.91 ug/cm²/min is the fifth smallest of six values in the table even though the relative humidity is the highest. Further investigation with tighter temperature and relative humidity controls are recommended. Placing the animal on warming pads and moistening the exposure sites at timed intervals prior to exposure are ways of controlling temperature and humidity. The temperature and relative humidity values in Table I were measured in the hood at the time of mustard vapor cap exposure.

Analysis of tape data. Table III contains the dpm measurements from the tape used to hold the vapor cap to the animal's back. The tape data demonstrated that saturated vapor within the cap diffused beyond the edges of the cap into the tape and that there was a large excess of HD available for uptake by the skin within the vapor cap. The dpm content of the tape was lower in study IV than in study I with the most dramatic decrease in studies V and VI. This decrease may reflect reduction in vapor cap adhesion during the studies or variation in tape size. Studies were carried out in different months: I (Dec), II (Dec), III (Feb),IV (Apr), V (May), and VI (Jun). An adhesion control could be introduced in which a vapor cap is taped to glass slide and this tape dpm compared to the tape from the animal study.

Validation of model. The polyethylene applicator cap contains 10 μl of HD. This quantity of HD in a vapor cap volume of 0.77 ml should achieve saturated vapor concentration of 1.4 μg/ml at an anticipated temperature of 30 ^{o}C[14], and there should be enough excess HD to allow for multiple use of the vapor cap. Studies I and II were designed to compare single through quadruple use of a vapor cap for HD application as follows. After the first animal exposure, the cap at position 4 (Figure 1) was replaced with cap A. The 7 used caps and cap A were applied to their respective sites on the second animal. Following the second animal exposure the position 3 cap was replaced with cap B, and after the third animal exposure the position 2 cap was replaced with cap C. Statistical analysis[15] of skin data in Table II for single through quadruple vapor cap application did not show significant differences between cap usage; however, the 5% power of this experiment was insufficeint to reject the null hypothesis of no differences. A larger study with more animals is necessary to clearly delineate optimum vapor cap usage.

Addition and Recovery. Recovery studies were performed on 8 sets of solutions as described in section A.5. The purpose of these curves was to isolate where in the processing of samples dpm losses might be occurring. The 8 curves produced are shown in Figures 3 and 4. A comparison of each curve's slope to the control slope of curve 1 was made. Curve 4 and 6 had

significantly smaller slopes than curve 1, $p<0.05$. Curve 6 represented the processing of skin samples. Corrections in skin dpm values were made using curve 1 to correct for ^{14}C losses in curve 6. These corrected values were used for C_T calculations in Table I. C_T decreased by 1.2% for study III (Table I) when corrected data was used vs uncorrected data while C_T values from the other studies had smaller changes.

Evaluation of site preference. Previous work[16] indicated possible preferential sites of HD uptake by the HGP based on microblister formation. A statistical evaluation[10] of the skin sample DPM data indicated that there was no preferential site of uptake of ^{14}C among the 8 sites chosen on the hairless guinea pigs used in this investigation.

SUMMARY

Penetration rates of saturated sulfur mustard in the HGP have been measured with ^{14}C-HD using 7-minute vapor cap exposures. The rates listed in Table I are in the range of 2 $\mu g/cm^2/min$. Penetration rate increased with relative humidity in five of six studies. Over a 24-hour period, the ^{14}C content of HGP skin samples revealed a sharp drop after 1 hour, becoming more gradual after 6 hours (Figure 2). Experiments with multiple applications of a vapor cap containing 10 μl of HD showed no significant differences between single and quadruple applications. Statistical analysis[15] of the dpm skin data in Table II indicates that a larger sample population will be required to determine significant differences in dpm data obtained from multiple applications of individual vapor caps. The ^{14}C content of dermal and epidermal samples at 24 hours post-exposure verifies binding of HD in these layers but not at sufficient activity to study the nature of this binding. Further studies will be needed to explain the change in ^{14}C content of the tape that held the vapor cap to the HGP. Quality of adhesive and extent of adhesion of double-sided tape to vapor cap and skin are possible explanations. Finally, additional studies should be carried out at exposure times of 5 and 10 minutes to investigate the effect of exposure time on ^{14}C-HD uptake by the HGP.

Figure 1

Animal Exposure Sites for HGP Back

Head

Pos 5	Pos 1
Pos 6	Pos 2
Pos 7	Pos 3
Pos 8	Pos 4

Tail

Figure 2

Rate Curve for ^{14}C-HD Cutaneous Uptake by HGP

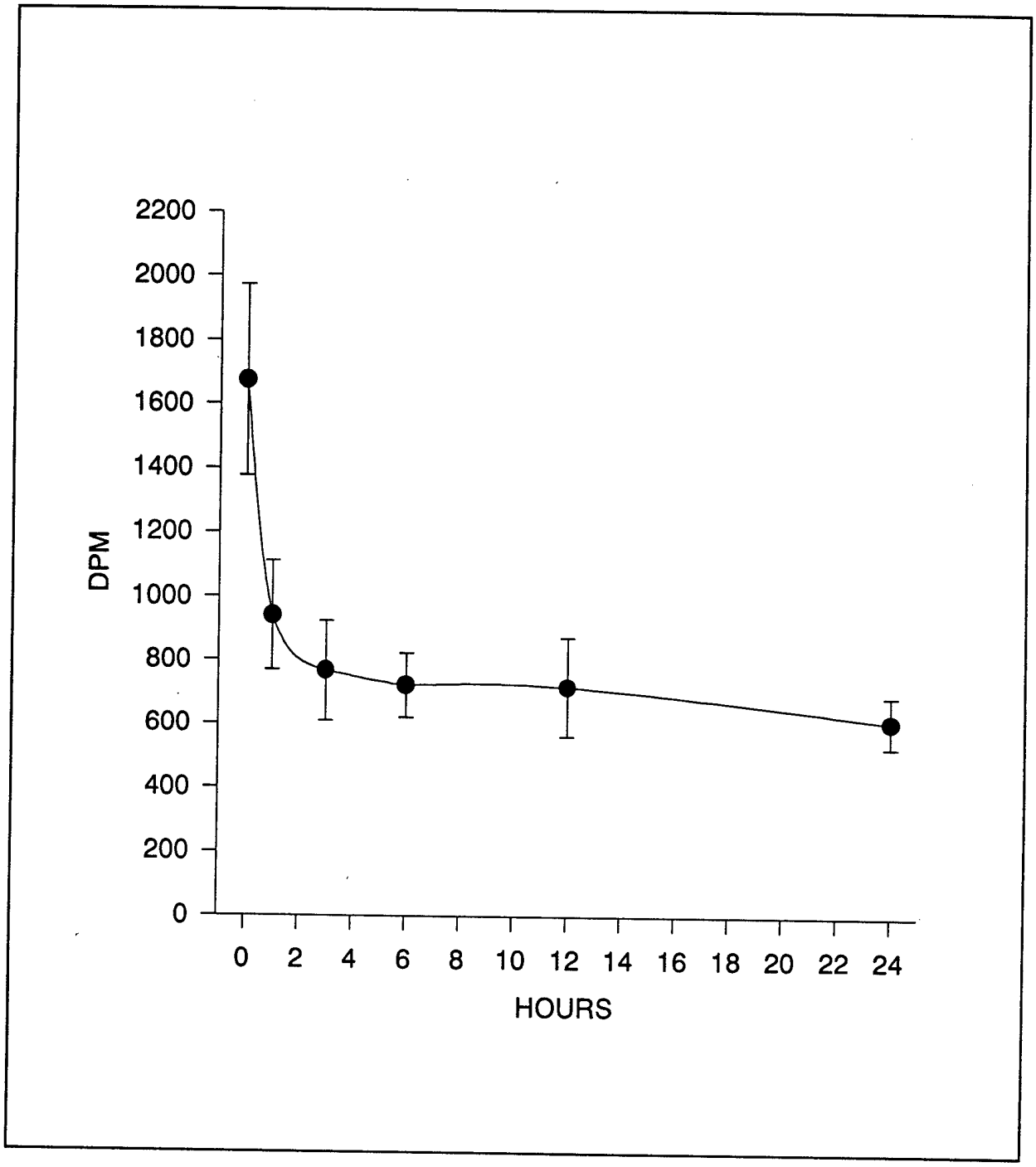

Figure 3

Addition and Recovery Curves 1-4

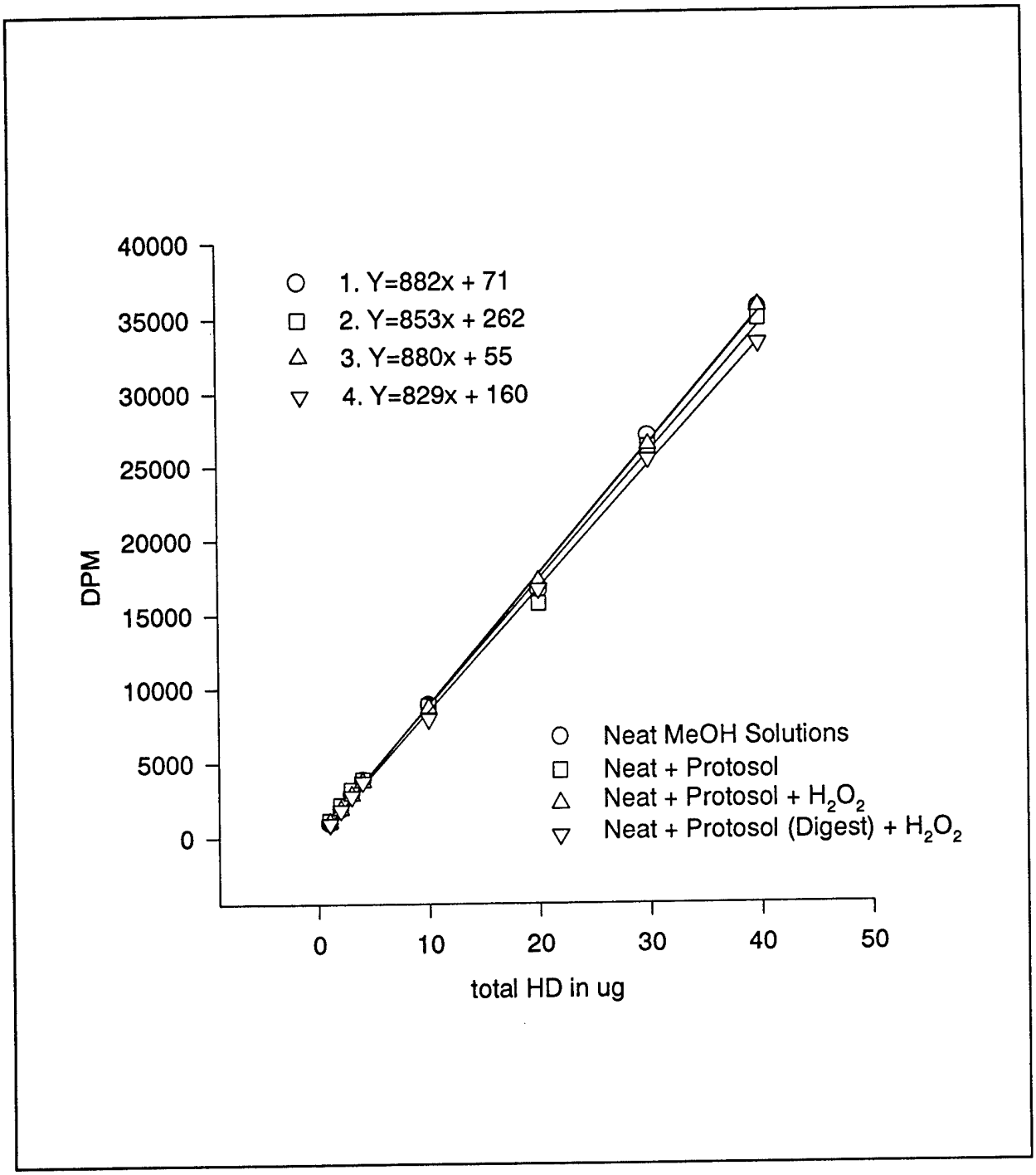

Figure 4

Addition and Recovery Curves 5-8

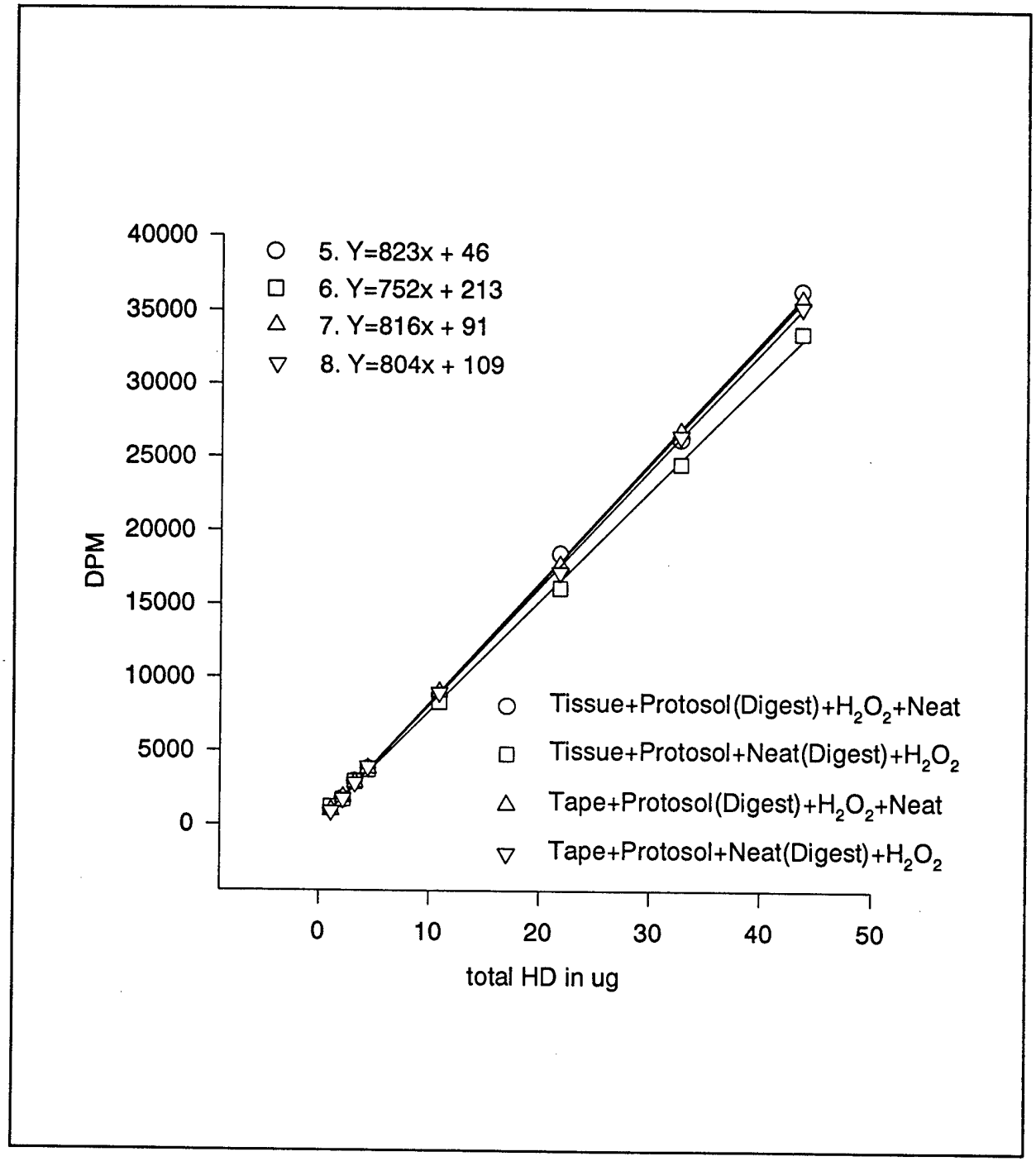

Table I

Results of Studies to Determine Concentration/Time (C_t) Values
for 7-minute HD Vapor Exposures from Zero-time HGP's

STUDY	ANIMALS n	TEMP (^{O}F)	RELATIVE HUMIDITY (%)	C_T ug/cm^2/min (SEM)*
I	4	60.6	59	2.27 (0.08)
II	4	62	36	2.06 (0.14)
III	3	64.4	17	1.61 (0.28)
IV	2	71.4	37	2.23 (0.31)
V	2	69.8	55	2.48 (0.16)
VI	2	66.2	66	1.91 (0.14)

*SEM, Standard Error of the Mean

Table II Skin Sample DPM

Study	Animal	MEAN*	S. D.	SEM
Zero Hour				
1	107	1861.0	216.8	76.7
1	108	1835.1	432.1	152.8
1	109	1878.3	306.2	108.2
1	110	2119.5	497.5	175.9
2	111	1764.8	395.5	139.8
2	112	1819.8	431.0	152.4
2	113	1400.0	281.1	99.4
2	114	1474.2	153.3	54.2
3	143	1810.2	373.1	131.9
3	144	1096.9	252.3	89.2
3	145	1169.8	197.3	69.7
4	212	1960.0	199.7	70.6
4	213	1491.6	313.5	110.8
5	220	2038.8	430.0	152.0
5	221	1803.1	480.1	169.7
6	239	1581.5	132.2	46.7
6	240	1361.5	148.5	52.5
One Hour				
3	149	951.0	124.0	43.8
3	150	759.2	80.6	28.5
3	151	702.4	137.9	48.7
6	241	1092.0	62.8	22.2
6	242	1066.7	163.8	57.9
6	243	1062.1	138.2	48.9
Three Hour				
3	146	1023.8	163.7	57.9
3	147	779.5	99.2	35.1
3	148	582.4	101.0	35.7
6	244	824.2	124.4	44.0
6	245	621.1	74.2	26.2
6	246	775.1	84.3	29.8
Six Hour				
3	140	635.9	95.4	33.7
3	141	812.1	123.9	43.8
3	142	620.2	89.2	31.5
4	209	688.2	97.1	34.3
4	210	877.7	96.8	34.2
4	211	697.1	105.7	37.4
Twelve Hour				
4	206	689.6	98.0	34.6
4	207	1006.6	84.6	29.9
4	208	771.7	214.3	75.8
5	217	589.0	64.0	22.6
5	218	612.2	160.3	56.7
5	219	636.1	93.6	33.1
Twenty four H o u r				
2	115	285.8	36.0	18.0
*2 1 1 5 E p i		258.7	85.9	43.0
*2 115Derm		121.1	13.5	6.7
2	116	385.2	53.6	26.8
*2 1 1 6 E p i		170.1	6.1	3.1
*2 116Derm		139.2	16.6	8.3
4	203	790.1	91.0	32.2
4	204	611.6	100.1	35.4
4	205	662.0	55.3	19.5
5	214	541.3	98.8	34.9
5	215	534.7	52.7	18.6
5	216	537.5	48.3	17.1
6	236	631.4	60.6	21.4
6	237	605.2	47.5	16.8
6	238	560.5	49.8	17.6

*n=8 for all animals except for 115 and 116 where n=4
** DPM data for skin punches separated into epidermal and dermal layers

Table III Tape Data DPM

Study	Animal	MEAN	S. D.	SEM
1.0	#107	8334.1	1194.6	422.3
1.0	#108	9523.6	1011.8	357.7
1.0	#109	10955.3	1060.3	374.9
1.0	#110	10644.9	1435.9	507.7
2.0	#111	8064.3	620.6	219.4
2.0	#112	8829.9	340.9	120.5
2.0	#113	8385.2	1264.7	447.1
2.0	#114	8055.4	738.2	261.0
2.0	#115	7885.5	818.2	289.3
2.0	#116	8034.3	620.3	219.3
3.0	#140	6129.4	2280.4	806.2
3.0	#141	8978.6	1318.7	466.2
3.0	#142	6694.3	2415.8	854.1
3.0	#143	7589.5	2063.7	729.6
3.0	#144	9458.0	1987.6	702.7
3.0	#145	5331.0	1608.9	568.8
3.0	#146	10396.5	696.4	246.2
3.0	#147	9937.9	750.9	265.5
3.0	#148	10630.2	770.7	272.5
3.0	#149	7892.2	1776.1	628.0
3.0	#150	9940.6	648.7	229.4
3.0	#151	6623.0	1973.6	697.8
4.0	#203	7585.5	2356.5	833.1
4.0	#204	5716.2	2693.7	952.4
4.0	#205	8129.6	1842.8	651.5
4.0	#206	6920.7	2773.1	980.4
4.0	#207	6770.0	2804.8	991.7
4.0	#208	8928.9	925.7	327.3
4.0	#209	6812.4	2133.8	754.4
4.0	#210	7283.8	2569.7	908.5
4.0	#211	6341.5	2236.9	790.9
4.0	#212	7213.1	1908.4	674.7
4.0	#213	9494.2	490.5	173.4
5.0	#214	6147.0	488.4	172.7
5.0	#215	5591.3	1021.7	361.2
5.0	#216	6074.3	1226.6	433.7
5.0	#217	5265.6	956.2	338.1
5.0	#218	6840.9	1094.4	386.9
5.0	#219	6511.7	1195.2	422.6
5.0	#220	7022.8	2130.1	753.1
5.0	#221	7528.6	1717.6	607.3
6.0	#236	2943.8	662.5	234.2
6.0	#237	2944.4	439.2	155.3
6.0	#238	3141.2	765.8	270.8
6.0	#239	3100.6	455.3	161.0
6.0	#240	3378.2	773.6	273.5
6.0	#241	3300.0	357.5	126.4
6.0	#242	3145.1	559.6	197.8
6.0	#243	4167.5	795.5	281.2
6.0	#244	3938.7	794.0	280.7
6.0	#245	3338.6	401.9	142.1
6.0	#246	4119.7	1035.4	366.1

Table IV

DPM Content of Vapor Caps

Study	1	2	3	4	5	6
n vapor caps	2	2	4	2	2	3
$\bar{x}$ measured value*	91735	83950	89118	82987	83801	81908
10$\bar{x}$ vapor cap content	917350	839500	891180	829870	838010	819080

* Measured at 1/10 dilution of actual content

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